

Nanotechnology-Based Electrochemical Sensors for Biomonitoring Chemical Exposures

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Focus of Presentation

- ▶ Need for direct reading exposure assessment methods
- ▶ Strategy for development & validation
- ▶ Example organophosphorus insecticides
 - Biomarker targets
 - Sensor development strategy
- ▶ Modeling strategy for interpretation of biomonitoring results
- ▶ Future directions & conclusions



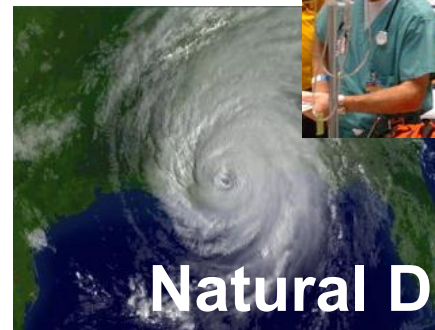
Occupational



Terrorism



Children



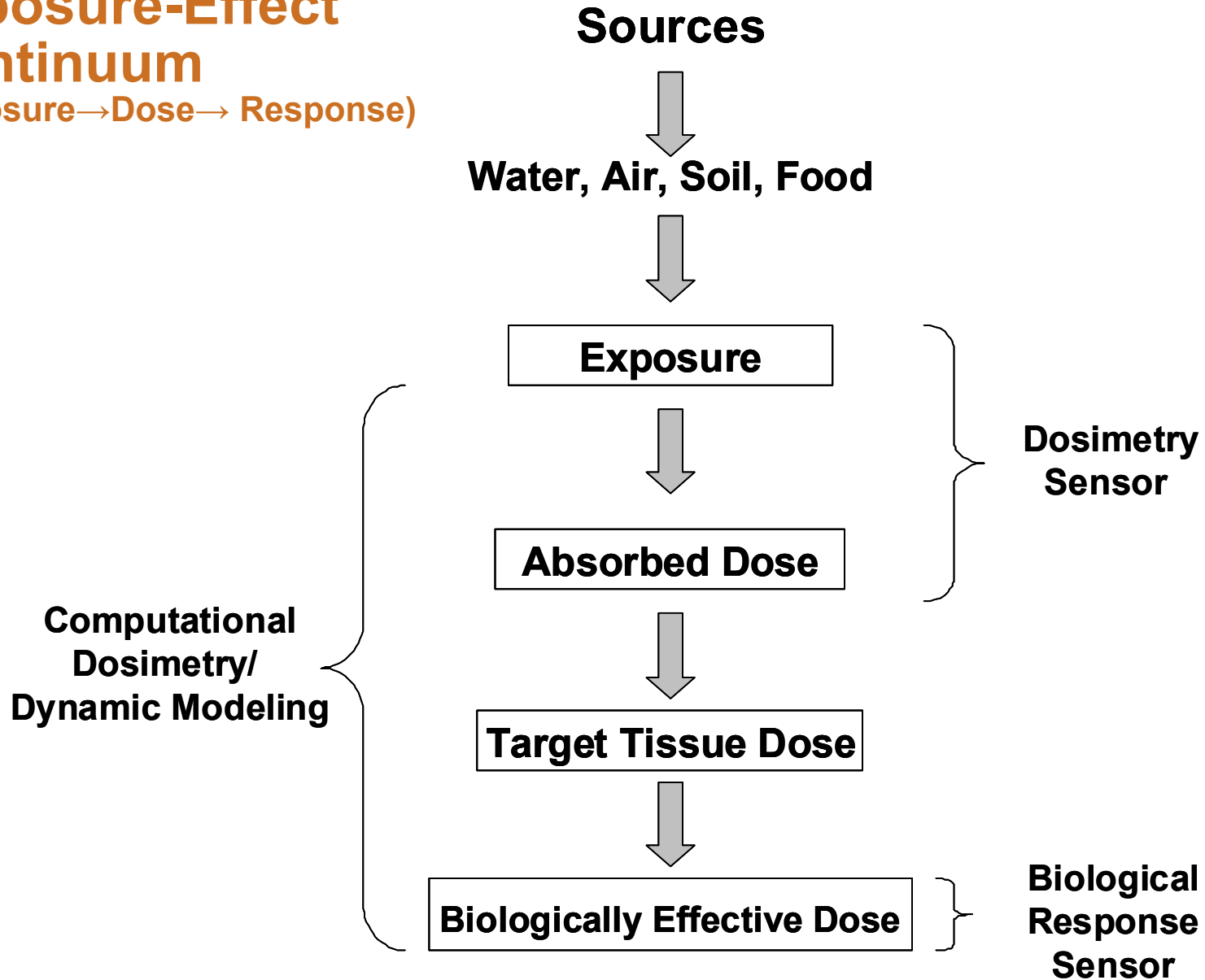
Natural Disasters



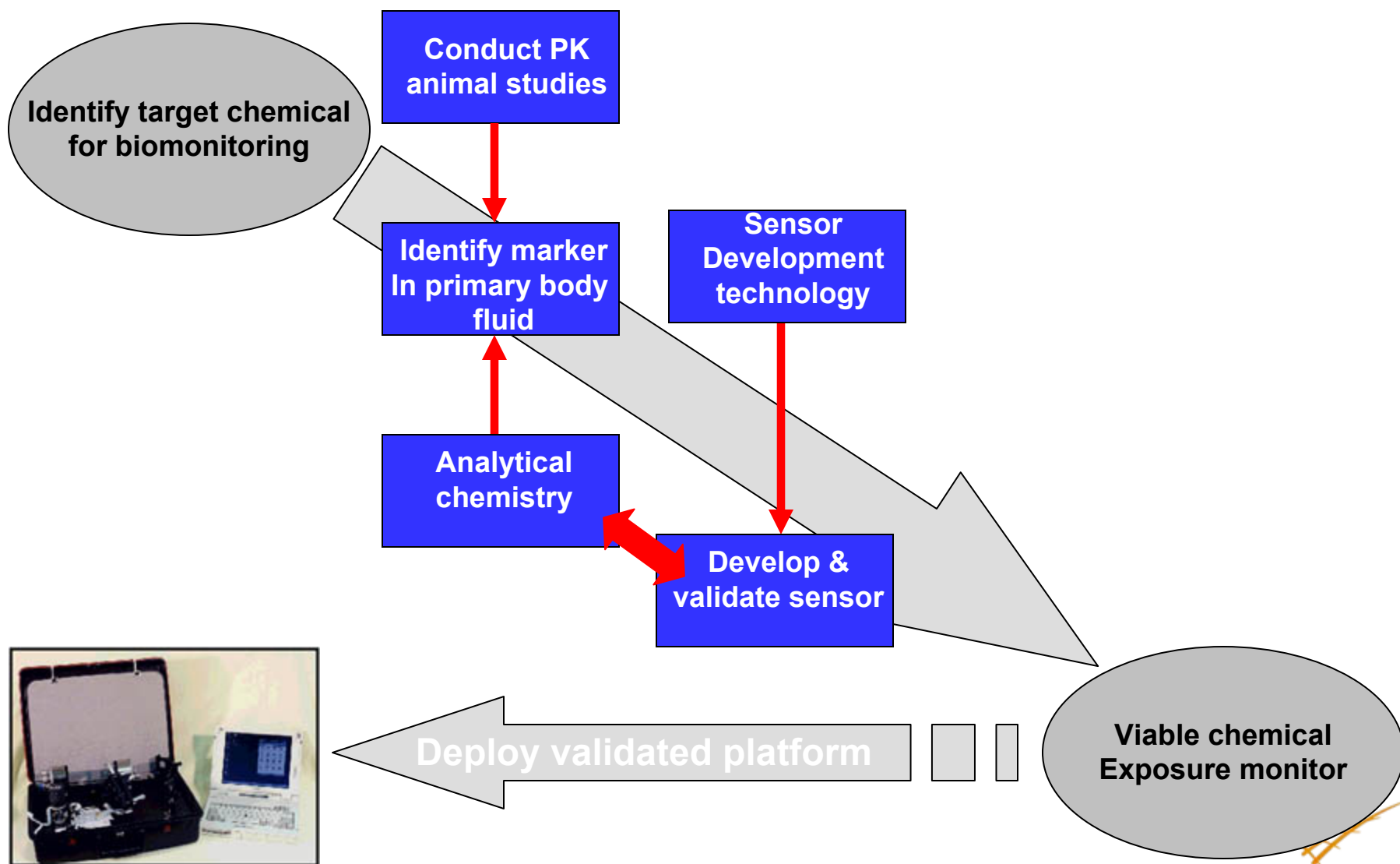
Pacific Northwest
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Exposure-Effect Continuum

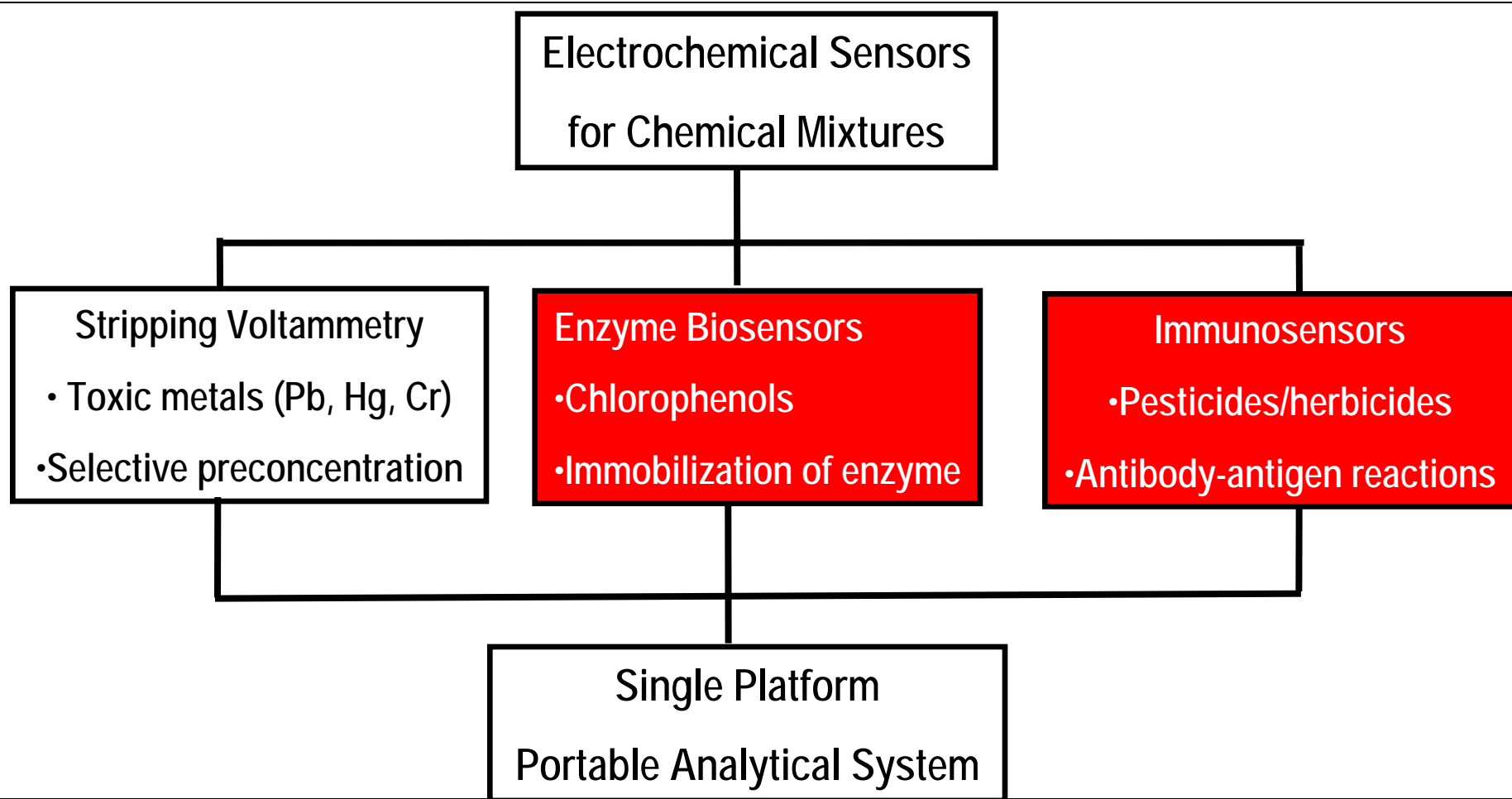
(Exposure→Dose→ Response)



Sensor Development Strategy



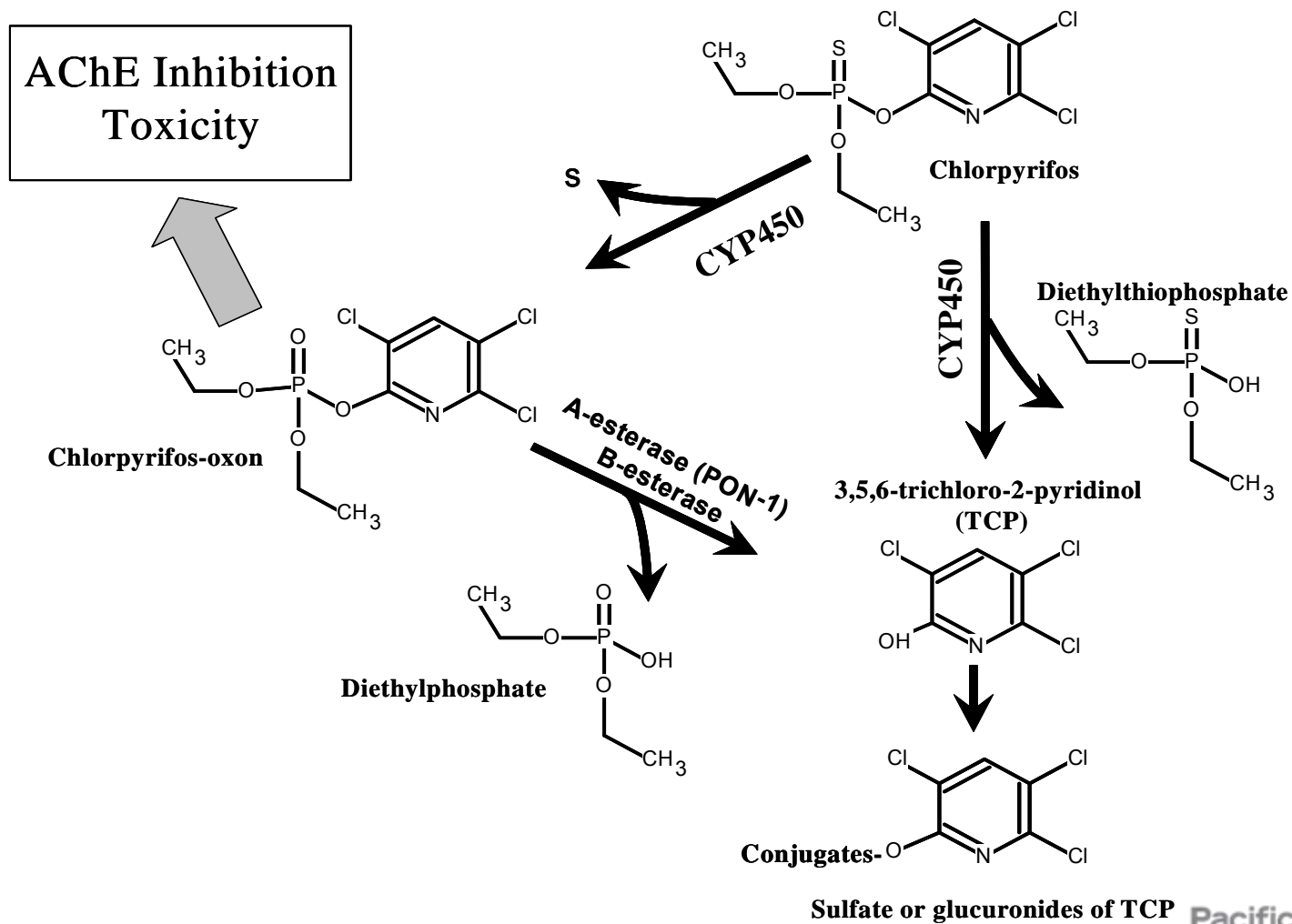
Sensor Development (Electrochemical)



Organophosphorus Agents (Pesticides & Chemical Warfare agents)

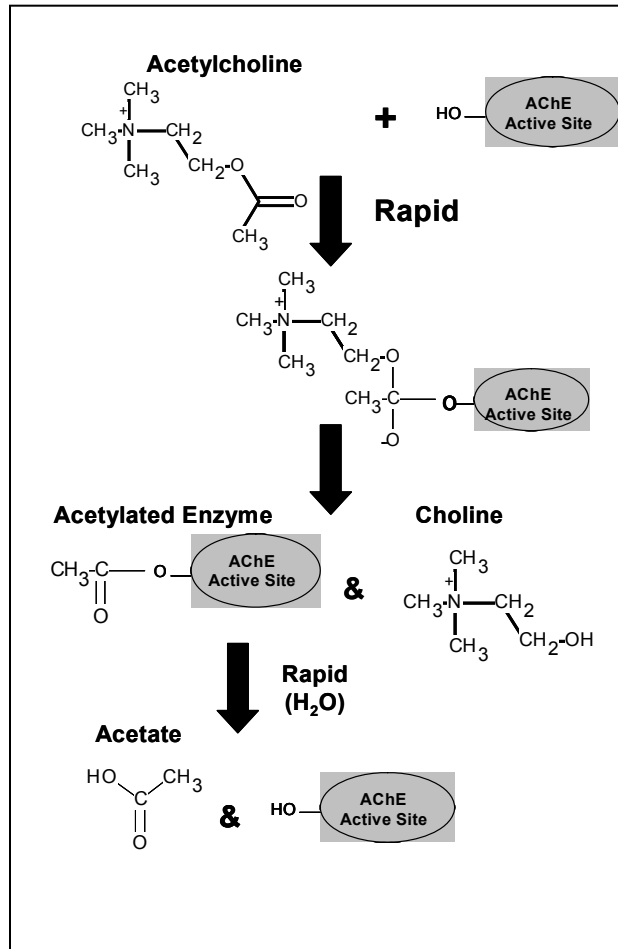
- ▶ Organophosphorus insecticide toxicity is associated with the inhibition of acetylcholinesterase (AChE) producing excess cholinergic stimulation.
 - Acute cholinergic crisis –SLUD/death
 - Chronic effects-delayed neuropathy
 - Children's sensitivity- neurodevelopmental effects
- ▶ Focus on organophosphorus insecticide biomonitoring
 - Cholinesterase inhibition (blood)
 - Metabolite measurement (urine)
 - Protein biomarkers (phosphorylated AChE)

Metabolism of Chlorpyrifos (Dursban®)

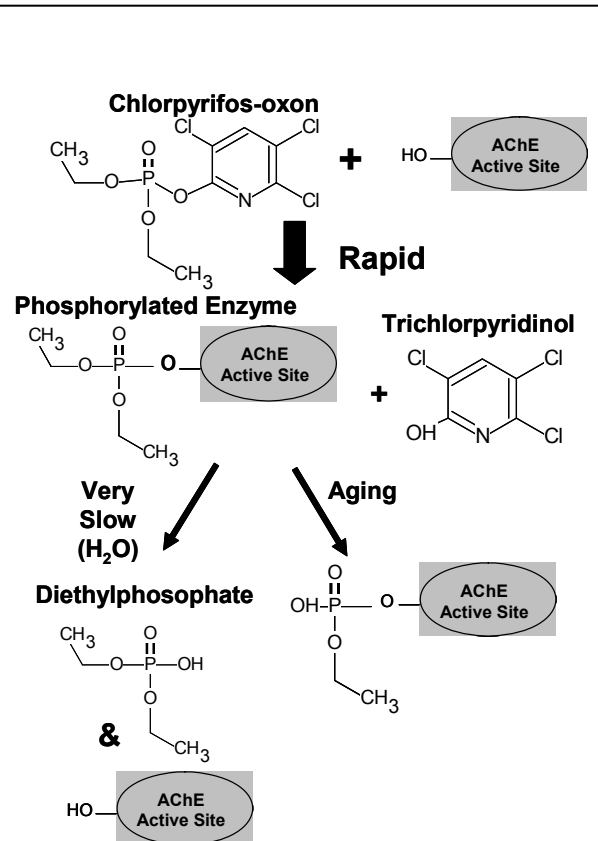


OP Pesticide Mode of Action: Cholinesterase (ChE) Inhibition

I. Acetylcholine



II. Organophosphates



Examples of: Organophosphorus Insecticide Sensor Platforms

Sequential/injection
immunoassay



Quantitation of pesticide
metabolite
(breakdown product)

Liu et al (2005)
Electrochem. Comm.,
7: 1463-1470.

Carbon nanotube-sensor
for enzyme activity



Quantitation of
cholinesterase activity

Wang et al (2008)
Environ. Sci Tech.,
42: 2688-2693.

Nano-particle
immunosensor
for phosphorylated AChE



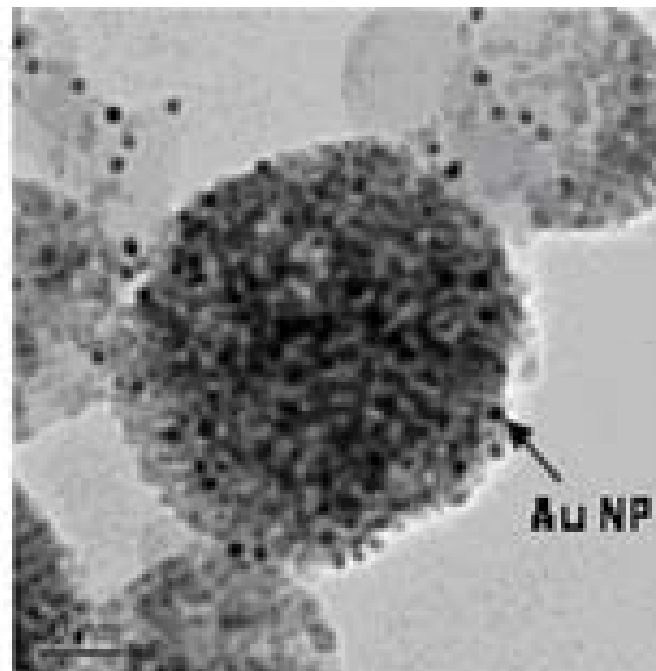
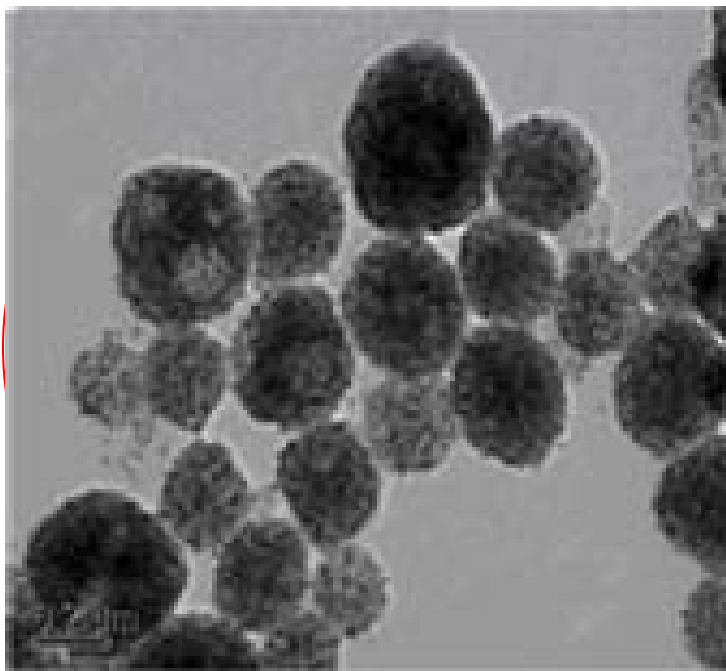
Quantitation of
modified AChE

Liu et al (2008)
Chem. Eur. J.,
(in press).

Sequential Injection/Electrochemical Immunoassay: TCP

- ▶ SIA system utilizes a thin layer electrochemical cell and permanent magnet to fix TCP antibody coated beads in reaction zone.
- ▶ Substrate solution + HRP-TCP → electroactive product.
- ▶ Monitored by square wave voltammetry.

Sequential Injection/Electrochemical Immunoassay: TCP

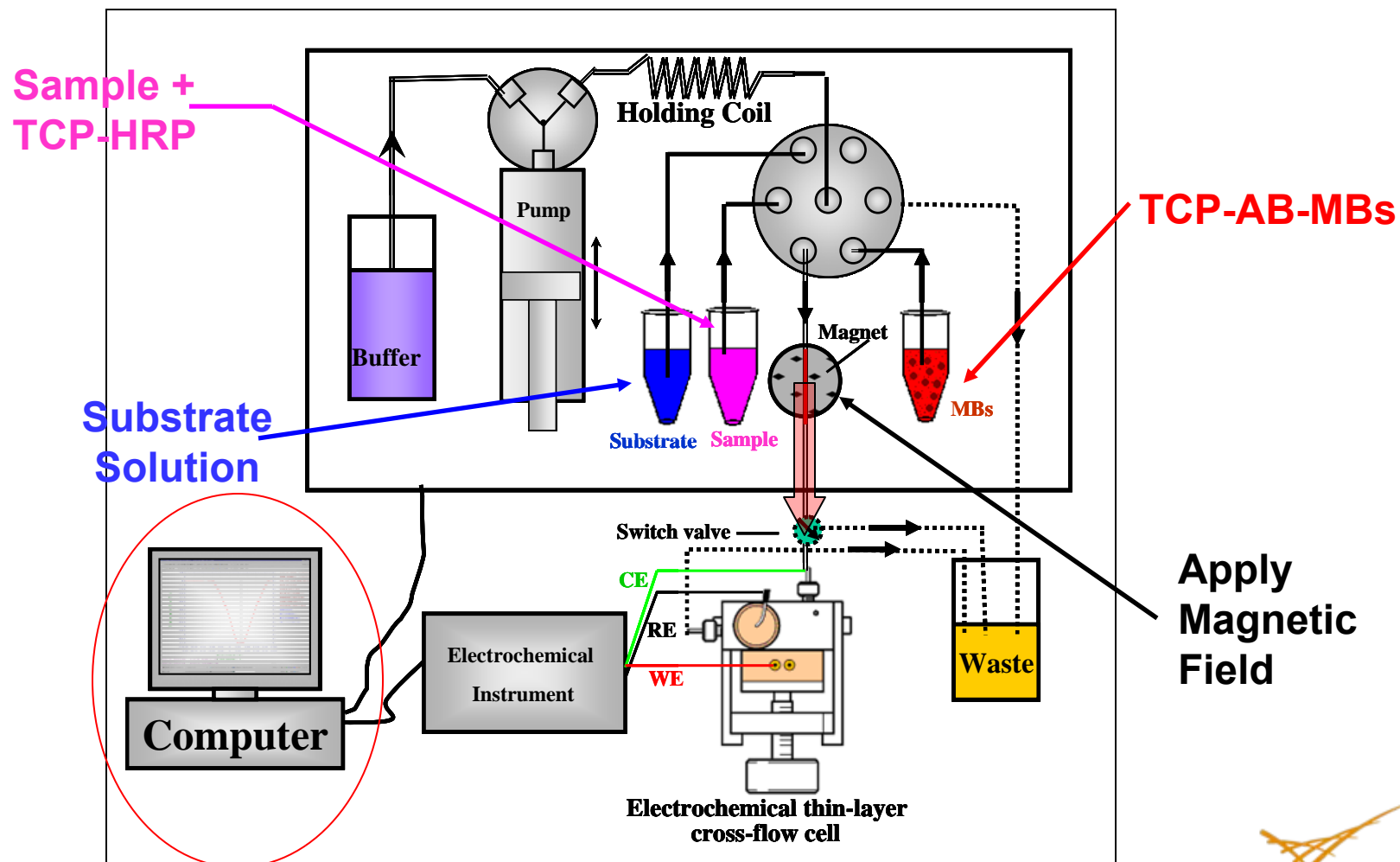


Thin Layer Flow cell
Electrochemical Detection

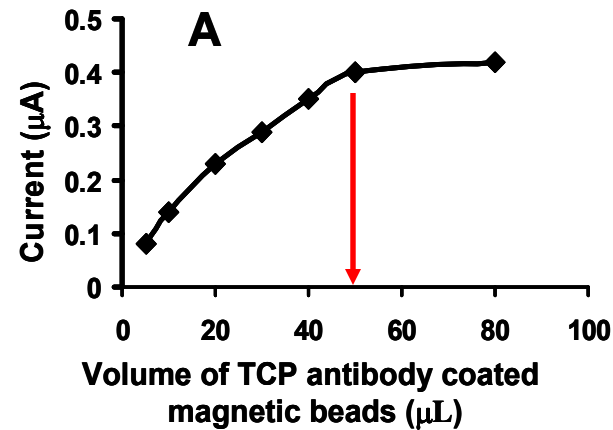
TEM- Magnetic Bead-gold nanoparticle assembly

Liu et al., 2005

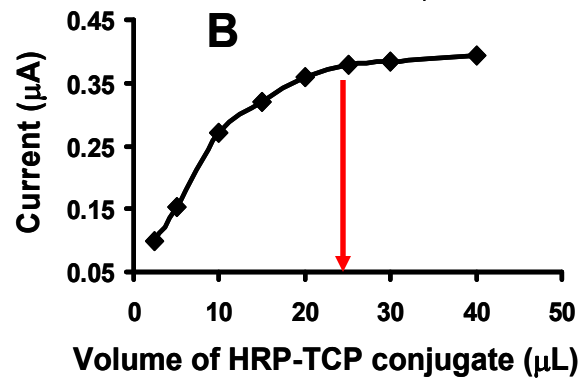
Sequential Injection/Electrochemical Immunoassay: TCP



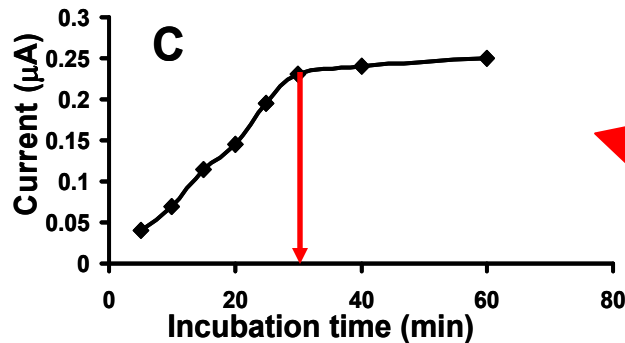
Optimization of Immunoassay Conditions



Impact of amount of MB on sensor response



Impact of HRP-TCP conjugate on sensor response

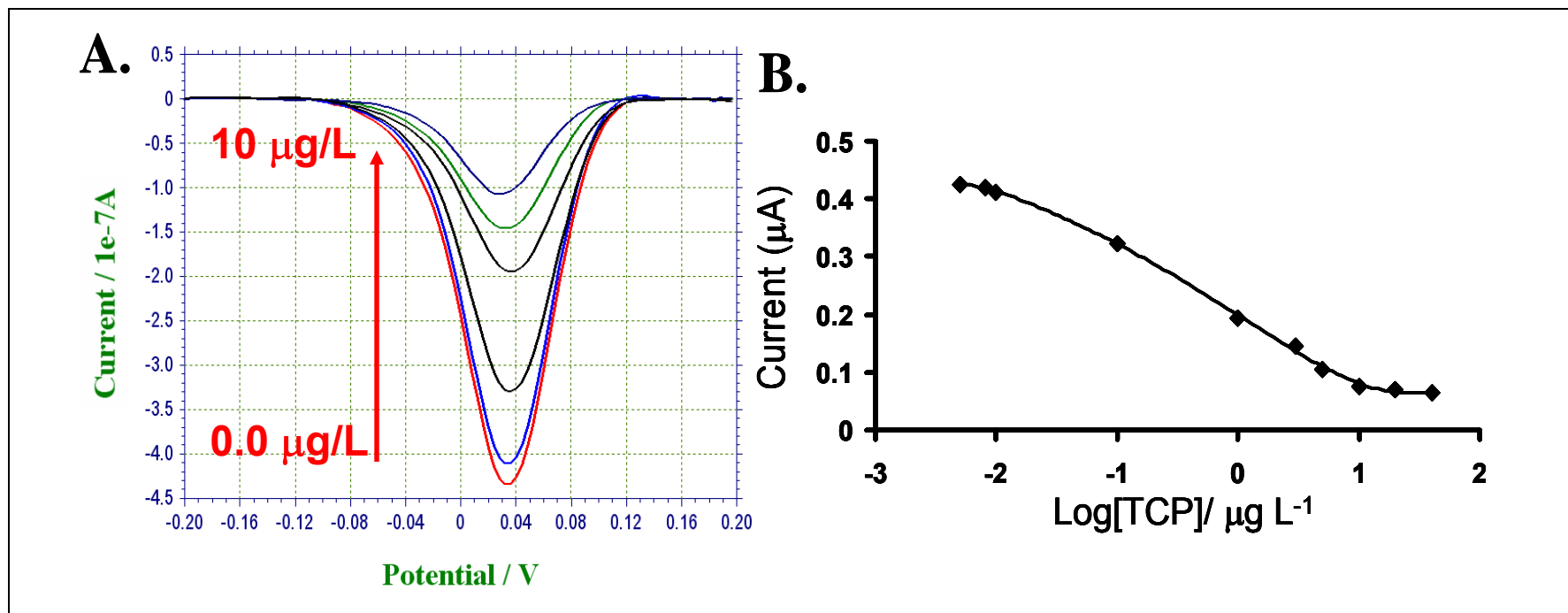


Impact of incubation time on sensor response

Liu et al., 2005

Analytical Performance

$$SWV_{signal} = \frac{[TCP - Ab - MBs]}{[TCP - HRP]} = \frac{1}{[TCP]}$$



Detection Limit – 6 ng/L (ppt) 50-fold lower than ELISA (250 ng/L)

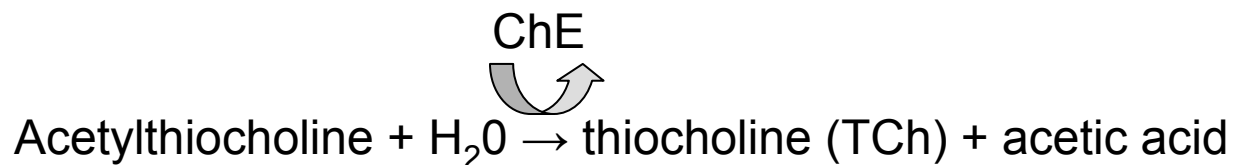
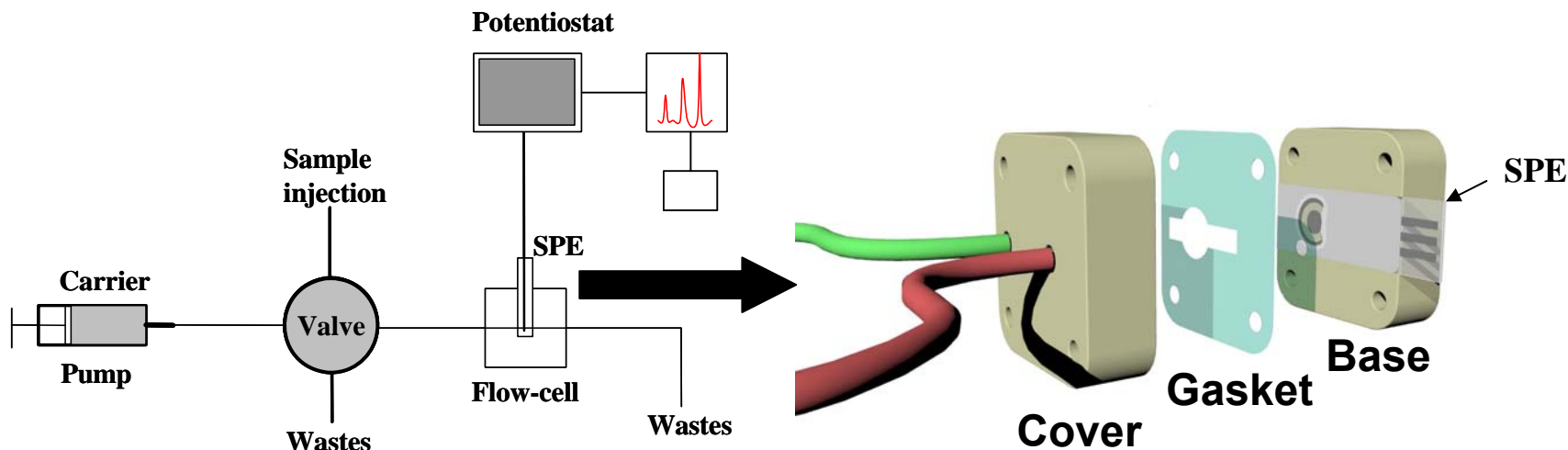
Summary: Sequential Injection/Electrochemical Immunoassay: TCP

- ▶ Parameters for SIA system optimized.
- ▶ Detection of TCP in low ppt range.
- ▶ Attractive approach for online immunoanalysis of aqueous systems without sample pretreatment.
- ▶ Ongoing *in vitro* studies will evaluate, refine and further optimize system for use with more complex biological matrices.

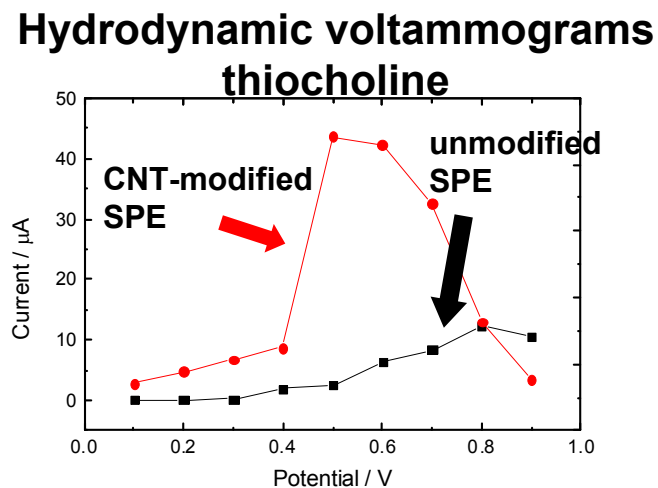
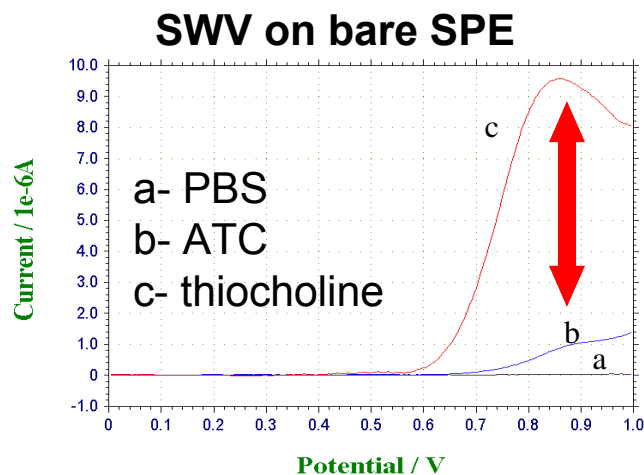
Carbon nanotube (CNT), screen-printed electrode (SPE) for ChE enzyme activity

- ▶ Electrochemical sensor based on carbon nanotube (CNT)-modified screen-printed electrode (SPE) integrated into a flow cell.
- ▶ Based on the excellent electrocatalytic activity of the CNT, the sensor detects electroactive species at high sensitivity and low potentials.
- ▶ Electrochemical techniques combined with flow-injection systems offers a simple and inexpensive approach for rapid onsite biomonitoring of enzyme activity.
- ▶ A disposable SPE was employed:
 - CNT-modified working electrode
 - Ag/AgCl reference electrode
 - Carbon ring counter electrode

Flow-Injection Sensing System for ChE Enzyme Activity Measurement

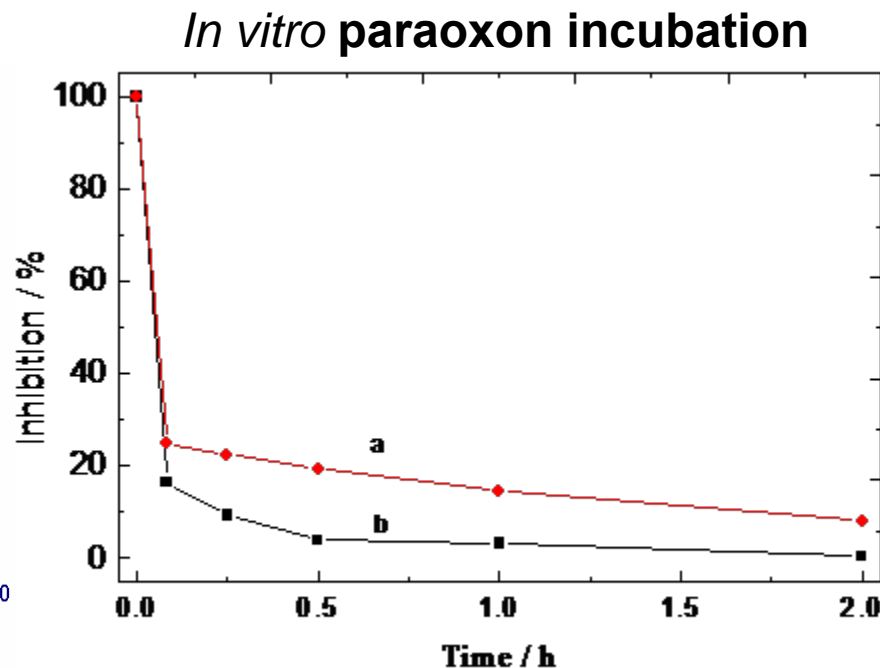
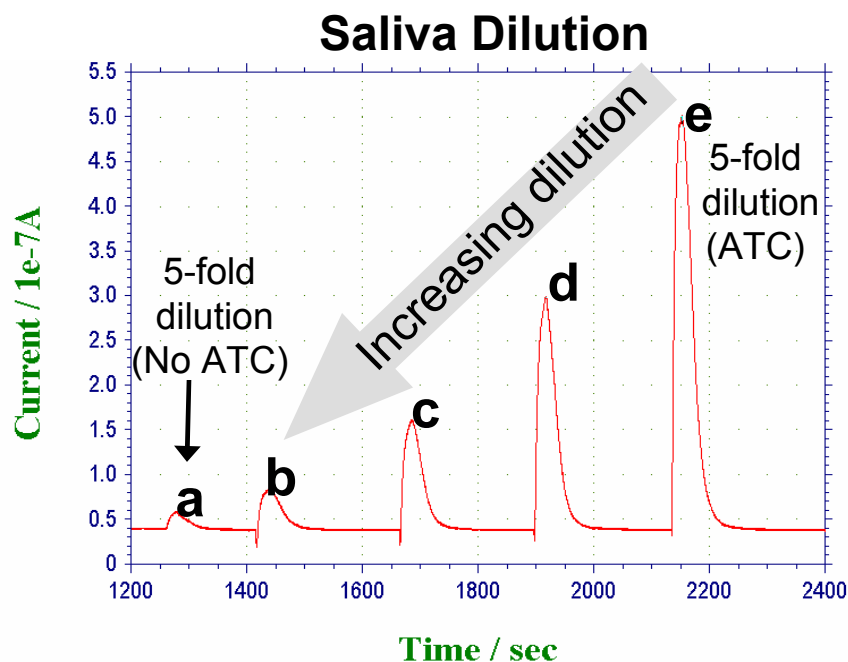


Electrochemical Characteristics of Thiocholine at the CNT-Modified SPE



- ▶ Current response for CNT-modified SPE is much higher.
- ▶ CNT can electrochemically catalyze the oxidation of the enzymatic product thiocholine, enhancing amperometric signal.

Rat Saliva ChE Response



- ▶ Saliva dilution from 5 to 40-fold, enzyme activity still detected (b→e).
- ▶ Low background (i.e. electroactive substances) in matrix (a).
- ▶ Paraoxon + 10-fold diluted saliva:
 - a) 0.7nM paraoxon (~80% inhibition at 0.5 hr)
 - b) 7 nM paraoxon (~97% inhibition at 0.5 hr)

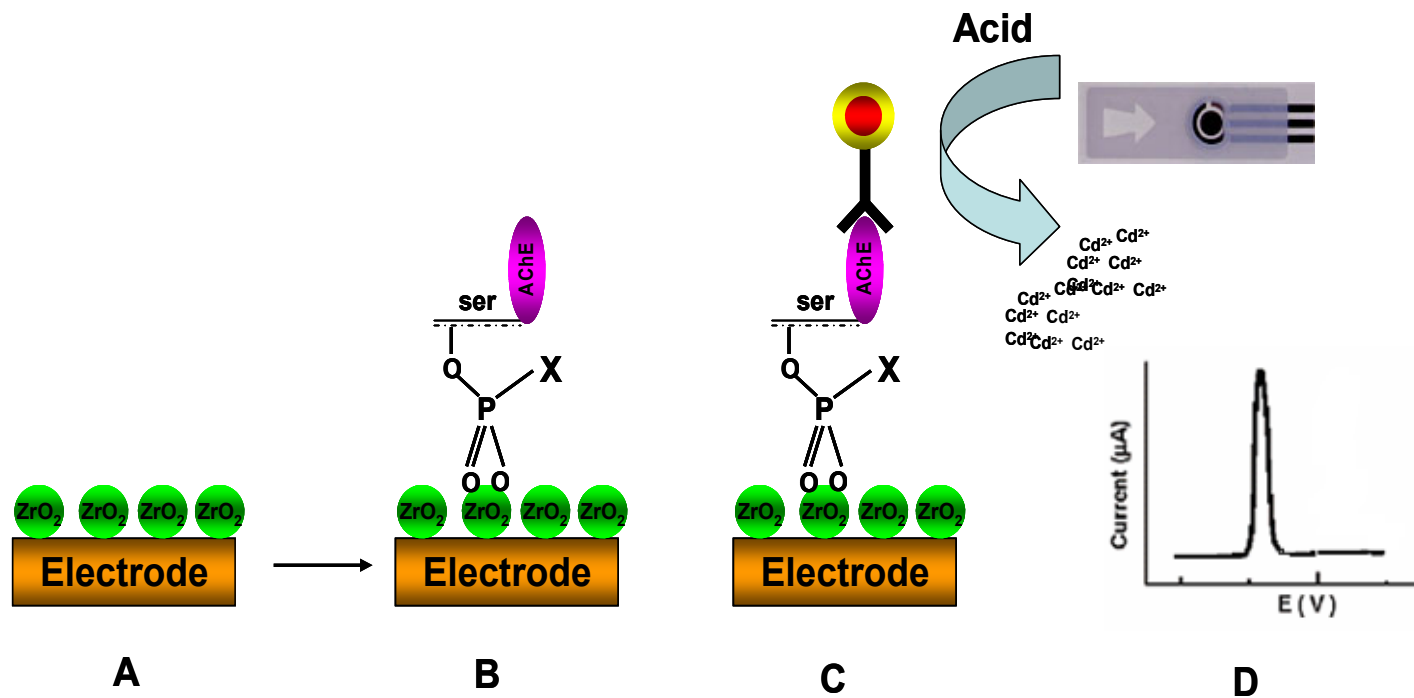
Summary: Carbon nanotube (CNT), screen-printed electrode (SPE) for ChE enzyme activity

- ▶ The CNT-based electrochemical sensor is simple, inexpensive, and sensitive for detecting ChE enzyme activity in saliva.
- ▶ Technique can be extended to other biological samples (i.e. plasma and RBC).
- ▶ *In vivo* animal validation studies will establish the sensitivity relative to more conventional assays (i.e. Ellman).

Nanoparticle-based electrochemical immunosensor for phosphorylated AChE

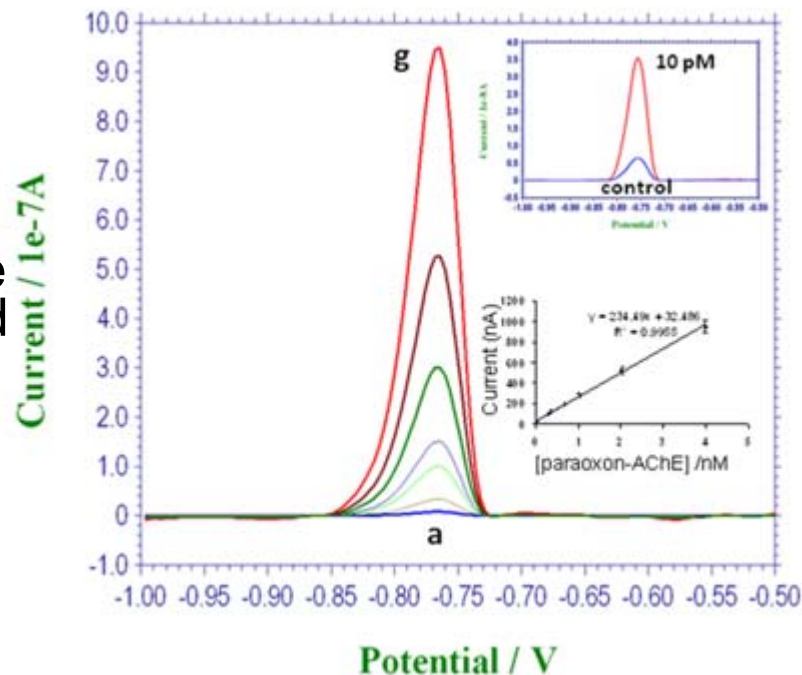
- ▶ Nanoparticle-based electrochemical immunosensor for detection of phosphorylated (i.e. inhibited) AChE.
- ▶ Zirconia (ZrO_2) nanoparticles used as selective sorbent for phosphorylated proteins.
- ▶ Quantum (QD) dots (CdS) used as tags to label monoclonal anti-AChE antibody to quantify immunorecognition event.
- ▶ Captured QD determined by SPE electrochemical stripping analysis (Cd) after acid-dissolution step.
- ▶ Initial studies focused on 'proof of principle' for detection of organophosphate-modified human AChE *in vitro*.

Quantum Dot Electrochemical Immunosensing: Phosphorlated AChE



Performance of the Immunosensor (optimal conditions)

- ▶ Monoclonal anti-AChE recognized both AChE & phosphorylated-AChE.
- ▶ Paraoxon utilized with human AChE to produce adduct.
- ▶ Performance evaluated over range (.01 nM \rightarrow 4 nM) of phosphorylated AChE concentrations.
- ▶ Human plasma AChE ~ 0.12 nM (8 ng ml⁻¹)
- ▶ A minimum 15% inhibition of plasma ChE would yield 0.018 nM and would be quantifiable.

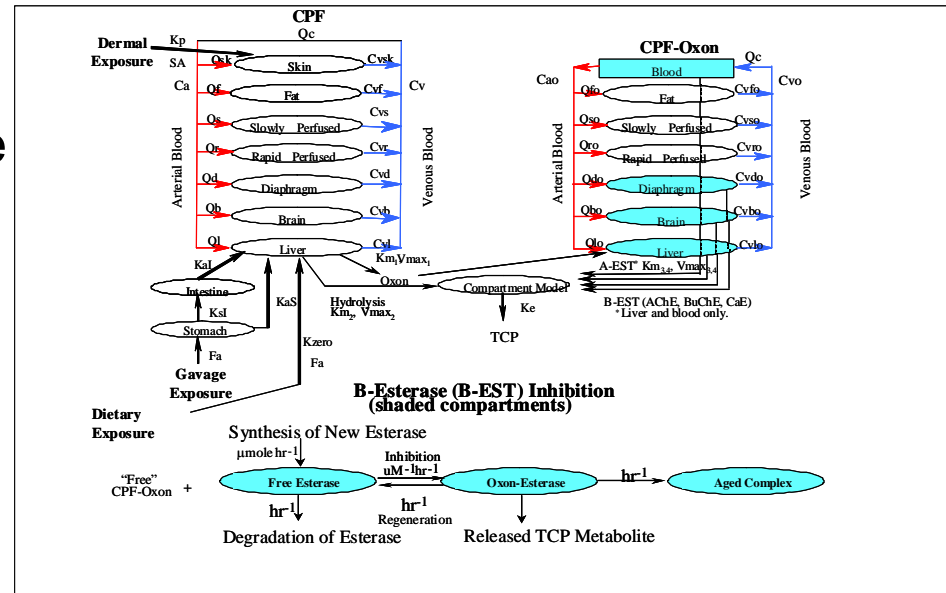


Summary: Nanoparticle-based electrochemical immunosensor for phosphorylated AChE

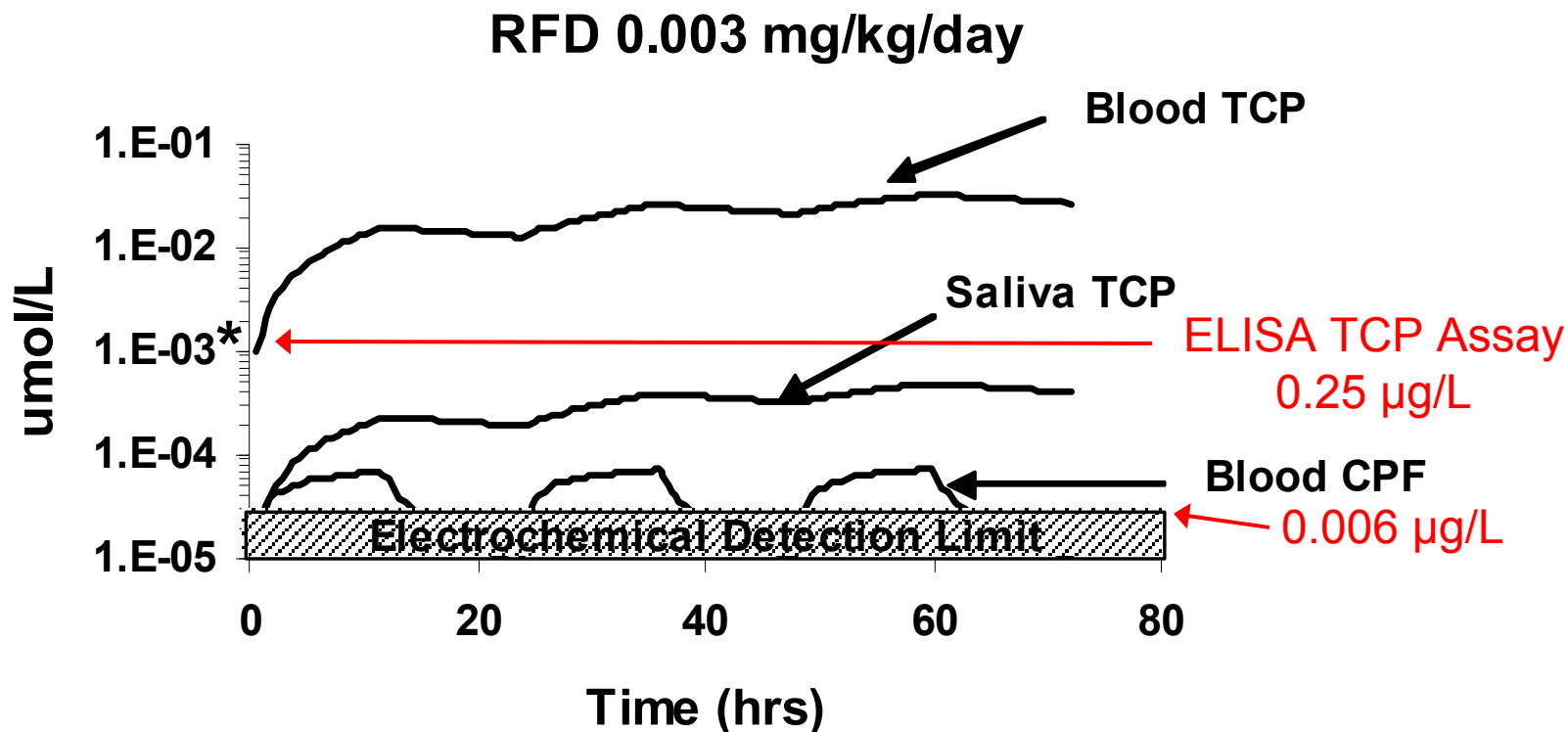
- ▶ Nanoparticle-based electrochemical immunosensor has potential to measure phosphorylated AChE as a biomarker.
- ▶ Demonstrated ability to detect phosphorylated AChE in complex biological matrices (data not shown).
- ▶ ZrO_2 shows promise as a good capture agent for phosphorylated proteins.
- ▶ Additional *in vitro* and *in vivo* validation studies are needed to establish performance criteria.

Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling

- ▶ PBPK/PD models have been developed for organophosphorus insecticides (chlorpyrifos, CPF).
- ▶ Capable of simulating target tissue dosimetry and biological response (i.e. ChE inhibition).
- ▶ Utility of model:
 - Cross species extrapolation (rat-human)
 - Integrates all routes of exposure (oral, inhalation, dermal)
 - Can simulate broad range of scenarios (repeated dosing, dietary intake, variable exposures)



Pharmacokinetic Analysis



Future Direction/ Conclusions...

- ▶ Laboratory validation studies
 - *In vivo* animals
 - *In vitro* human tissue
- ▶ Engineer into field deployable unit
- ▶ Design and conduct field validation studies

Conclusion

The development of portable nanotechnology-based electrochemical sensors has the potential to meet the needs for ***low cost, rapid, high-throughput*** and ***ultrasensitive*** bioassays for biomonitoring an array of chemical markers.

Acknowledgements

- | | |
|----------------------|------------------------------|
| ▶ Yuehe Lin PhD | -Sensor development |
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